

**Exhibit A**

## Background of Invention

Beef cattle producers in the United States have experienced a narrowing margin of profit from their businesses. Profit is a measurement of positive return over expenses. To increase profit one must cut expenses and/or increase return. According to Peterson et al. (1987) feed accounts for 70% of operation cost in beef cattle production. Late summer and early fall are periods of time when dams have high metabolic requirements which must be met by declining forage quality. By early weaning calves from spring calving cows, the dams have the ability to increase BCS prior to winter and decrease cost of feed without detrimental effects on calf performance. Peterson et al. (1987) found that cows with early weaned (EW) calves consumed 45.3% less hay (TDN basis) than did cows with traditional weaned (TW) calves. He reported that EW calves gained more than the TW calves from time of EW to TW and were heavier at TW than TW calves. Therefore, EW pairs were 43% more efficient at converting TDN into calf gain. Fluharty et al. (1996) found that EW calves had heavier weights, higher efficiencies and better dressing percentages than TW calves. The EW calves exhibited more back fat, better quality grades, and yield grade was not affected.

## Summary of Invention

The single-calf heifer system (SCH) is a program designed to allow a heifer to produce one calf prior to harvest. This experiment increases value of heifers of non-replacement status by incorporating them into the SCH. Although these culls are considered inferior to the replacement counterparts, they may have been considered superior prospects 30 yr. ago (Brethour and Jaeger, 1989). Brethour and Jaeger (1989) also stated that many heifers are culled due to large body frame leading to high maintenance requirements. However, in the SCH these animals are profitable because of faster growth during feedlot period and larger, more profitable carcasses. Reiling et al. (1995) reported that heifers fed a high-energy diet could efficiently produce weight gain and milk simultaneously under feedlot conditions. The key to this system is for the heifers to reach harvest weight by 30 mo in order to qualify for top carcass price. Brethour and Jaeger (1989) found that the SCH had more carcass bone maturity and slightly higher shear force values as compared to their non-pregnant contemporaries. However, the USDA yield and quality grades were comparable and acceptable.

Integrating early weaning with the single-calf heifer system may generate profit by decreasing feed costs of herd dams and increasing value of cull heifers. The Integrated System (IS) may allow non-replacement heifers to produce a calf by 18 mo of age and a carcass by 24 mo of age. This IS is similar to the traditional SCH however the period from weaning to harvest is shortened. Feedlot ration allows greater, faster gains resulting in younger puberty age. Early puberty permits early insemination and is a key to the IS allowing for shorter days on feed as well as avoidance of carcass maturity problems.

Sexed semen is a relatively new technology that grants the IS ability to perpetuate the program using the female progeny of the initial heifers. Schenk and Seidel (1999) reported that sexed semen could alter the sex ratio to >85% based on DNA content using flow cytometer/sperm sorter. They also reported that AI using low dose sexed semen resulted in pregnancy rates in heifers that were 70-90% of unsexed traditional dose controls. Additional aspects as discussed in PCT Publication No.WO 96/12171, hereby incorporated by reference.

Integration of early weaning, sexed semen, and single-calf heifer systems has the potential to create extra revenue for a producer by increasing value of non-replacement heifers while simultaneously decreasing feed costs to herd dams. By accomplishing the previous, the IS has the potential to generate profit.

#### **Brief Description of the Drawings**

Figure 1 is a time line showing a treatment sequence according to one embodiment.

Figures 2 and 3 show weight gains for heifers managed by one embodiment of the Integrated System (IS) according to one embodiment.

#### **Detailed Description of the Preferred Embodiments**

Experimentation was conducted at the CSU Eastern Colorado Research Center near Akron, CO. Eighty-six Red Angus X Hereford heifers were split into two treatment groups. Forty-six heifers of non-replacement status were early weaned at  $110 \pm 15.0$  d of age and managed in the IS. The other 40 heifers (TW) were weaned at  $229 \pm 2.8$  d of age and managed in a Traditional Replacement System (TRS).

The EW heifers were placed into a feedlot directly after early weaning and on self feeders for 140 d then bunk fed for the remainder of a phase I feedlot period. The ration ingredients included triticale grain, sunflower meal pellet, corn, ground alfalfa and Rumensin®. Weight of the EW heifers was measured every month and the ration evaluated and adjusted according to heifer gain. The TW heifers were weaned 107 d after the EW heifers. At the time of weaning of TW heifers, BCS of dams of the EW and TW heifers was recorded as well as weight of the TW heifers. The TW heifers were weighed again at AI of EW heifers.

Behavior and physiological indicators monitored puberty of the EW heifers. First, electronic Heat Watch® system monitored behavioral patterns and the onset of standing heat via mounting by other cattle. Three androgenized cows were commingled with the EW heifers to aid in the process of heat detection. Puberty was also monitored physiologically by progesterone (P<sub>4</sub>) assay of blood taken via jugular aspirations at 10 day intervals for a period of two months prior to MGA/PGF synchronization and again 10 d prior to and on day of PGF<sub>2</sub>  $\alpha$  injection. The percent of TW heifers at puberty was measured by P<sub>4</sub> assay for one month prior to MGA/PGF synchronization of EW heifers. Heifers were considered pubertal when serum P<sub>4</sub> concentration was greater than 1 ng/ml in a 10 d period.

Estrus synchronization of the EW heifers was accomplished by top dressing feed with MGA for 14 d followed by PGF<sub>2</sub>  $\alpha$  injection 19 d after last day of MGA feeding (Figure 1). Heifers were synchronized at  $250 \pm 15.0$  d of age. Heifers were AI'd following standing heat up to 72 hr post PGF<sub>2</sub>? injection according to a.m./p.m. protocol. At 72 h post PGF<sub>2</sub>? injection, fixed-time mating of all remaining pubertal heifers occurred (Figure 1). A 45 d breeding period allowed heifers three opportunities to be AI. All rebreeds were based on standing heat recorded by Electronic Heat Watch® and/or visual observation.

The IS produces all female progeny to perpetuate the system in subsequent years by utilizing sexed semen. Semen donors were three Red Angus bulls with low birth weight EPD's. Semen was sorted using flow cytometry, selecting X-chromosomal sperm (Schenk and Seidel, Jr., 1999). Semen doses for insemination contained three million sperm per dose and at least 35% post-thaw motility. A first service conception rate of EW was determined using ultrasonography 34 d post fixed insemination.

Statistical analysis completed using general linear model (GLM) procedure of SAS (1988) and when appropriate, means were separated using Tukey's HSD (SAS 1988).

Forty-six heifers of non-replacement status were early weaned at  $110 \pm 15.0$  d of age at  $141 \pm 21.1$  kg. Forty heifers of replacement status were traditionally weaned at  $229 \pm 2.8$  d of age at  $262 \pm 25.40$  kg. At time of TW, EW heifers were  $202 \pm 15.0$  d of age and  $249 \pm 6.8$  kg. The TW were  $27 \pm 12.2$  d of age older ( $P < .0001$ ) than the EW heifers and had greater weights at time of TW. However, the EW heifers had greater WDA than the TW heifers,  $1.2 \pm .10$  kg and  $1.1 \pm .11$  kg ( $P < .0001$ ) respectively. From EW to TW, the dams of the EW heifers had greater BCS than dams of TW heifers,  $6.6 \pm .80$  and  $5.8 \pm .78$  ( $P < .0001$ ), as a result of lactation ending and allowing for increased biological utilization of grazing forage nutrition. The dams of the EW heifers were put on winter range without additional supplementation under weight-loss management conditions. The dams of the TW heifers were also put on winter range managed to maintain and or gain BCS. It is believed that during the winter period, the dams of the TW heifers will require supplementation and will be an economic benefit, therefore favoring early weaning due to reduced feed cost. However, due to the mild winter in Akron, CO in 1999-2000, the economic benefit will be understated as very little supplement was needed regardless of weaning strategy.

The EW heifers 28 d weight gains throughout the feedlot phase varied from  $.86 \pm .371$  kg/d to  $2.00 \pm .367$  kg/d with an overall average of  $1.25 \pm .139$  kg/d (Figures 2 and 3). These variations in 28 d gains are attributed to adjusted feed rations to allow for gains that would induce early puberty. Once the heifers began to cycle, the ration was backed down to avoid over-fattening of heifers and possible negative impacts on subsequent reproduction/calving difficulty. During phase I, three heifers were taken off the study prior to breeding. One heifer died shortly after weaning due to unknown cause, the second heifer had poor performance and negative gains, and the third heifer foundered. These losses may have been due to aggressive feeding during the early weaning period and will be accounted for in the final economic analysis.

Puberty was reached at various ages dependent on the individual heifer. One month prior to synchronization, 20% of EW heifers were pubertal compared to 8% of the TW heifers. This induction of early puberty is contributed to nutrition allowing greater gain and weight of the EW heifers. At time of PGF injection, the number of EW heifers cycling increased to 85% with only 7 heifers pre-pubertal. The EW heifers ( $248 \pm 15.0$  d of age) were much heavier than TW heifers ( $314 \pm 2.8$  d of age) at this time,  $313 \pm 28.0$  kg and  $293 \pm 31.4$  kg ( $P < .0001$ ). Based on 500 kg mature weight of herd dams, the EW and TW heifers had reached approximately 63% and 57% of mature weight respectively.

Heifers that became impregnated to sexed semen on the first service of AI were 27% of those cycling and fixed-time mated. The low conception rate may be due to any one or a combination of low fertility of the first estrus cycle, low numbers of semen in a dose and properties of sexed semen.

Phase I of the integration of early weaning, sexed semen and single-calf heifer systems achieved increased BCS of dams; accomplished satisfactory gain performance of heifers which enabled induction of early puberty and resulted in 9 mo old heifers impregnated to sexed semen. Unsatisfactory first service conception rates using sexed semen are a concern for the system; however, the opportunity for three services of AI may result in adequate overall pregnancy rate. Exploration of the complete system including and extending beyond phase I will achieve a better analysis of the IS potential to increase value of cull heifers.

As can be easily understood from the foregoing, the basic concepts of the present invention may be embodied in a variety of ways and for various species, with or without alterations as appropriate. For example, although discussed in a bovine context, other species may also be managed. Additionally, although the SCH concept may obviously not apply, overall management and individual aspects may be generally applicable to swine and others as well. Further, the invention also should be understood as involving both management techniques as well as separable individual elements to accomplish the appropriate system or subsystem. In this application, the management techniques are disclosed as part of the results shown to be achieved by the various systems described and as steps which are inherent to the system. They include the results of utilizing the systems as intended and described. Importantly, as to all of the foregoing, all of these facets should be understood to be encompassed by this disclosure.

The discussion included in this provisional application is intended to serve as a basic description. The reader should be aware that the specific discussion may not explicitly describe all embodiments possible; many alternatives are implicit. It also may not fully explain the generic nature of the invention and may not explicitly show how each feature or element can actually be representative of a broader function or of a great variety of alternative or equivalent elements. Again, these are implicitly included in this disclosure. Where the invention is described in process oriented terminology, most elements of the process can implicitly involve devices to achieve that process. Method claims may not only be included for the process described, but also apparatus claims may be included to address the devices which achieve these processes. Neither the description nor the terminology is intended to limit the scope of the

claims which will be included in a full patent application.

It should also be understood that a variety of changes may be made without departing from the essence of the invention. Such changes are also implicitly included in the description. They still fall within the scope of this invention. A broad disclosure encompassing both the explicit embodiment(s) shown, the great variety of implicit alternative embodiments, and the broad methods or processes and the like are encompassed by this disclosure and may be relied upon when drafting the claims for the full patent application. It should be understood that such language changes and broad claiming will be accomplished when the applicant later (filed by the required deadline) seeks a patent filing based on this provisional filing. The subsequently filed, full patent application will seek examination of as broad a base of claims as deemed within the applicant's right and will be designed to yield a patent covering numerous aspects of the invention both independently and as an overall system.

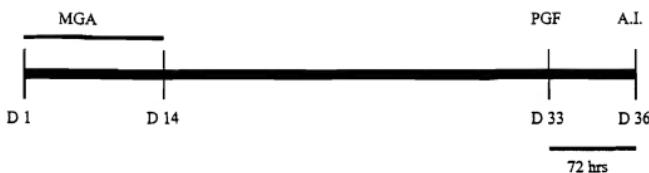
Further, each of the various steps of the invention may also be achieved in a variety of manners. This disclosure should be understood to encompass each such variation, be it a variation of an embodiment of any apparatus embodiment, a method or process embodiment, or even merely a variation of any element of these. Particularly, it should be understood that as the disclosure relates to elements of the invention, the words for each element may be expressed by equivalent apparatus terms or method terms -- even if only the function or result is the same. Such equivalent, broader, or even more generic terms should be considered to be encompassed in the description of each element or action. Such terms can be substituted where desired to make explicit the implicitly broad coverage to which this invention is entitled. As but one example, it should be understood that all actions may be expressed as a means for taking that action or as an element which causes that action. Similarly, each physical element disclosed should be understood to encompass a disclosure of the action which that physical element facilitates. Regarding this last aspect, as but one example, the disclosure of a "synchronization element" should be understood to encompass disclosure of the act of "synchronizing" -- whether explicitly discussed or not -- and, conversely, were there only disclosure of the act of "synchronizing", such a disclosure should be understood to encompass disclosure of a "synchronization element" and even a means for "synchronizing". Such changes and alternative terms are to be understood to be explicitly included in the description.

Any publications or other references mentioned in this application for patent, as well as all

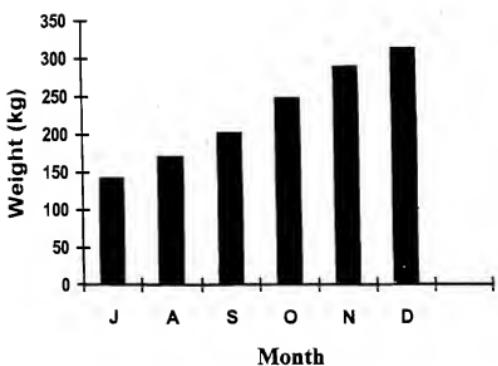
references listed in the list of References To Be Incorporated By Reference In Accordance With The Provisional Patent; or other information statement filed with the application are hereby incorporated by reference; however, to the extent that such information or statements incorporated by reference might be considered inconsistent with the patenting of this/these invention(s) such statements are expressly not to be considered as made by the applicant(s). Thus, the applicant(s) should be understood to claim at least: i) each of the herd-management methods as herein disclosed and described, ii) the individual methods disclosed and described, iii) similar, equivalent, and even implicit variations of each of these devices and methods, iv) those alternative designs which accomplish each of the functions shown as are disclosed and described, v) those alternative designs and methods which accomplish each of the functions shown as are implicit to accomplish that which is disclosed and described, vi) each feature, component, and step shown as separate and independent inventions, vii) the applications enhanced by the various systems or components disclosed, viii) the resulting products produced by such systems or components, and ix) methods and apparatuses substantially as described hereinbefore and with reference to any of the accompanying examples, and x) the various combinations and permutation of each of the elements disclosed.

## ABSTRACT

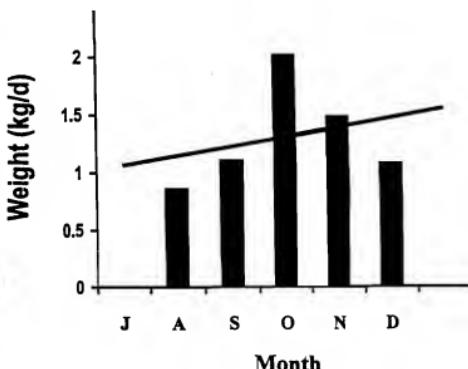
Integrating early weaning and sexed semen into a single-calf heifer system may be an alternative to traditional marketing of non-replacement heifers. This integrated system (IS) has potential for heifers to produce a calf by 20 mo of age and a carcass by 24 mo of age. Phase I of the IS may include early weaning, estrous synchronization, and AI. Sexed semen can be utilized to yield female progeny, decrease calving difficulty risk and create a second generation to perpetuate the IS. The IS group in one example was composed of 46 Red Angus X Hereford heifers rejected for replacement status and early weaned at  $110 \pm 15.0$  d. The control group was 40 traditional weaned (TW) heifers from the same herd, managed in a traditional replacement system (TRS). The early weaned (EW) heifers were placed on self-feeders and adjusted to a finishing ration. They were fed to 65% of mature weight to induce early puberty by 9 mo of age. At time of TW, age of EW heifers was less than TW heifers ( $202 \pm 15.0$  d and  $229 \pm 2.8$  d respectively,  $P < .0001$  ). There was no difference between groups in weight at TW, yet EW heifers had greater weight per day of age (WDA) than TW heifers,  $1.2 \pm .10$  kg and  $1.1 \pm .11$  kg ( $P < .0001$ ). Dams of EW heifers had greater body condition score (BCS),  $6.6 \pm .80$  than TW dams,  $5.8 \pm .78$  ( $P < .0001$  ) by TW. At AI of EW heifers, weight was greater for EW than TW heifers,  $313 \pm 28.0$  kg and  $293 \pm 31.4$  kg respectively ( $P < .0031$  ) and WDA of EW and TW heifers were  $1.2 \pm .10$  kg/d and  $1.0 \pm .10$  kg/d ( $P < .0001$  ) One month prior to MGA/PGF protocol synchronization, 20% EW and 8% TW were exhibiting estrous cycles as determined by progesterone assay. At fixed-time mating, age of EW heifers was  $293 \pm 15.0$  d with 85% cyclic. Ultrasonography at 35-d post AI revealed first service conception rates of 27%. Phase I of the IS increased BCS of dams, enabled greater gains and weights of heifers, induced early puberty and resulted in 9 mo old heifers pregnant to sexed semen.



**FIG. 1**



**FIG. 2**



**FIG. 3**